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Non-Destructive Global and Localized 2D T_1/T_2 NMR Relaxometry to Resolve Microstructure in Apples Affected by Watercore

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Abstract Apples can be considered as having a complex system formed by several structures at different organization levels: macroscale ($>100\ \mu\text{m}$) and microscale ($<100\ \mu\text{m}$). This work implements 2D T_1/T_2 global and localized relaxometry sequences on whole apples to be able to perform an intensive non-destructive and non-invasive microstructure study. The 2D T_1/T_2 cross-correlation spectroscopy allows the extraction of quantitative information about the water compartmentation in different subcellular organelles. A clear difference is found as sound apples show neat peaks for water in different subcellular compartments, such as vacuolar, cytoplasmatic and extracellular water, while in watercore-affected tissues such compartments appear merged. Localized relaxometry allows for the predefinition of slices in order to understand the microstructure of a particular region of the fruit, providing information that cannot be derived from global 2D T_1/T_2 relaxometry.

Keywords Non-destructive · Microstructure · NMR · Localized · Global · Watercore

Introduction

Food can be considered a complex system formed by several structures at different organization levels. The most general level divides the structure into macrostructure (above $100\ \mu\text{m}$) and microstructure (below $100\ \mu\text{m}$) (Aguilera 2012). Macrostructure, which is formed by smaller structures

(microstructures), affects the macroscopic properties, such as rheological behaviour, transport properties and organoleptic attributes, such as texture or in-mouth sensations perceived by the consumer. It is also related to the distribution of gases, water and connectivity and mobility through the pores (Hills et al. 1996; Vittadini et al. 2005). All these properties will affect the stability of food products. These are some of the reasons why the food industry is currently dealing with the study of the microscopic level and its relation to the macroscopic scale.

In the particular case of fruit, the study of microstructure can help when extending shelf life by applying drying processes for preservation (Prothon et al. 2001; Mujumdar and Law 2010; Fernandes et al. 2011; Pei et al. 2013) or storage for large periods in a controlled atmosphere (Herremans et al. 2013a). The latter is of the utmost importance for fruit, which may be affected by some disorders. This is the case with apples potentially affected by watercore. Watercore is a physiological disorder characterized by water-soaked, glassy regions in the fruit flesh around the core line. Several authors have identified two distinct forms of watercore: block and radial (Clark et al. 1998; Harker and Watkins 1999; Melado-Herreros et al. 2013a). In mild cases, watercore symptoms may disappear during storage, but in more severe cases when the fruit is stored for a long time, it may undergo anaerobiosis and develop alcoholic flavours or suffer internal breakdown and browning (Bennedsen and Peterson 2005; Dart and Newman 2005). Watercore symptoms are also related to a metabolic change in sugar composition (Bowen and Watkins 1997; Yamada et al. 2006; Melado-Herreros et al. 2013a), which makes watercore apples especially appreciated in some regions of Asia and Spain. It is regarded as an added value because of their sweet and juicy flesh (Dart and Newman 2005; Kasai and Arakawa 2010; Melado-Herreros et al. 2013a).

Recent studies have used X-ray and micro-X-ray CT for apple microstructure study (Mendoza et al. 2010; Verboven

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et al. 2008; Ting et al. 2013; Herremans et al. 2013a, b). This technique allows the microstructure elucidation with resolution of a few micrometres. It visualizes the cell walls, the void networks and the microporosity tissues. However, these methods provide a small field of view (approximately 1 mm^2), which makes the information provided insufficient for describing the entire fruit with heterogeneous structures (Musse et al. 2010). Furthermore, this technique requires taking samples from the fruit, which makes the study of the whole fruit impossible.

Nuclear magnetic resonance (NMR)-based techniques are successful for monitoring microstructure in food. In fact, for some fruit disorders, such as internal browning in pears and watercore disorder in apples, magnetic resonance imaging (MRI) methods provided better contrast than X-ray CT (Lammertyn et al. 2003; Herremans et al. 2014). It is possible to use MRI microimaging, which typically works in the range of $5\text{--}40 \text{ }\mu\text{m}$ (Hills 1998). The advantage of using MRI is that it makes the measurement of the spatial distribution of porosity possible, and it allows the estimation of microporosity on intact, whole fruit, thanks to its large field of view. Musse et al. (2010) determined apple and tomato microporosity by combining multiple spin-echo images to assess transversal relaxation rate, R_2 ($R_2=1/T_2$), and multiple gradient-echo images to assess effective transversal relaxation rate, R_2^* ($R_2^*=1/T_2^*$), to quantify microporosity. In complex structures, such as complex foods, MRI methods lack the capability to resolve detail at the nano- and meso-scale. An alternative is a combination of NMR protocols based on relaxometry and diffusometry, together with the spatial information that provides MRI (Van As and Van Duynhoven 2013). Zhang and McCarthy (2013) used spin-spin relaxation time (T_2) and MRI quantitative analysis to study pomegranate quality attributes. T_2 showed correlation with soluble solid content, while MMR image statistical features and soluble solid content correlation were poor. Oztop et al. (2014a) used multi-slice-multi-echo images combined with the analysis of the decay (NMR relaxometry) to assess water and fat distribution in chicken nuggets coated with two different formulations (methylcellulose and control) at four different frying times (0, 1, 2 and 4 min) and at two different frying temperatures. They obtained a relaxation time distribution of different regions of the nugget (from the core and the crust) and correlated relaxation times and relative areas of the proton pools in crust and core regions with moisture and fat content. Oztop et al. (2014b) monitored the release and water uptake of divalent cations (Mn^{+2} and Ca^{+2}) in whey protein-based gels, applying multi-slice spin-echo sequence and T_2 NMR relaxometry. Adriaensen et al. (2013) used an optimized multiple spin echo (MSE) MRI sequence for the assessment of multi-exponential transverse relaxation in fruit tissues. They tested their sequence in a phantom and in actual food: apple and tomato, and they could distinguish microstructure at subcellular levels. Nevertheless,

such a sequence has to be optimized with regard to the characteristics of the tissue to be examined.

The knowledge of a watercore-affected apple's microstructure is of major interest in order to apply the appropriate postharvest treatment, such as an adequately controlled atmosphere for storage and for selling the product at the correct moment and to the correct market. To the best of our knowledge, a large number of pieces of work on watercore microstructure do not exist. Herremans et al. (2014) performed high-resolution scans using X-ray CT of small parts of both healthy and affected tissues that were physically dissected from the fruit. From visual inspection of the micro-CT images, they found that the pores between cells are void in healthy tissue (porosity 30 %) characterized by numerous isotropic connections. In affected tissue, the porosity decreases to 7 %, and the pore diameter is also reduced, resulting in a more dense tissue compared to the healthy regions. Though this study covers the intercellular space, the watercore subcellular area remains unexplored. Exploration can be performed using relaxation methods, thanks to NMR techniques.

Some authors have used NMR relaxation and diffusion methods in order to acquire information from the subcellular level. Hills and Remigereau (1997) studied the changes in subcellular water compartmentation in the parenchyma of small pieces of apple tissue during drying and freezing. They concluded that non-spatially resolved NMR relaxation and diffusion techniques provided the best results for the study of subcellular water compartmentation in tissue compared to conventional MRI. They found out that mild air drying in a fluidized bed results in loss of water from the vacuolar compartment but not from the cytoplasm or cell wall regions. They concluded that in the freezing process, the vacuolar compartment is the first to freeze. Nevertheless, 2D cross-correlation relaxation methods have enormous potential for characterizing complex systems such as food. These types of experiments combine the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and the inversion recovery (IR) sequence. Thus, each peak in the 2D spectrum is characterized by a particular proton longitudinal and transverse relaxation time (T_1 and T_2 , respectively), which differs according to the local water content and the size of the pore compartment, structure and tissue integrity (Hills 2010). These types of experiments give information on water transport through the microstructure (Marigheto et al. 2005). Hernández-Sánchez et al. (2007) performed a microstructure study in small pieces of pear tissue using T_1/T_2 correlation spectra. They obtained information about the cell structure integrity. On sound fruit, it was possible to distinguish different compartments for the vacuole and the cytoplasm, while in damaged pears, both compartments tended to merge, pointing to disintegration of the cell structure.

In any case, 2D T_1/T_2 relaxometry was a sequence designed at low magnetic field strength. Such sequences have

been implemented in the high field in previous studies (Hernández-Sánchez et al. 2007; Melado-Herreros et al. 2013b) on small food tissues and food models, respectively. Nevertheless, they had never been applied on whole actual food before. This study intends to show the successful implementation of such sequences for the microstructural study of watercore in apples. The importance of using such sequences at high magnetic field strength relies on the possibility of detecting the microscopic diffusion, produced by the magnetic susceptibility changes (Marigheto et al. 2008). Also, the bore size is important since commercial relaxometers working at low magnetic field strengths have a small size, which does not permit the study on whole fruit but only on small tissue samples (Hernández-Sánchez et al. 2007; Marigheto et al. 2008). Thanks to the implementation to high field, studies on whole fruit can be accomplished, which would permit the performance of non-destructive and non-invasive microstructure studies of food.

Traditionally, 2D T_1/T_2 global relaxometry has been particularly valuable in samples having microscopic heterogeneity, such as cellular plant tissue in which relaxation time peaks from water at different subcellular compartments, metabolites and biopolymers can be resolved. Nevertheless, complex materials, such as fruit, display heterogeneity on both microscopic and macroscopic distance scales. This means that when global 2D T_1/T_2 relaxometry is applied for investigating subcellular changes, only an average relaxation spectrum of the whole fruit is obtained. As an alternative, one could physically dissect each layer from the fruit. However, using 2D T_1/T_2 localized relaxometry solves the problem, as it is possible to study several slices of the fruit without destroying the sample (Venturi and Hills 2010). In this study, we will study both global (applied on the whole fruit) and localized (applied at certain slices of the fruit, without dissecting the sample) relaxometry sequences in order to study in a non-destructive way the microstructure of watercore disorder, in terms of the composition of tissues at subcellular scale.

The objective of this work is the implementation of 2D T_1/T_2 global and localized relaxometry sequences at a high magnetic field on a whole food product, in this case apples, in order to perform an intensive, non-destructive and non-invasive microstructure study of apples affected by watercore.

Materials and Methods

Samples

An apple cultivar was selected for this work due to its susceptibility to watercore development. The apples were grown and harvested during the 2012 season at an experimental orchard in Estación Experimental de Aula Dei (CSIC, Zaragoza, Spain). In early October, a batch of 72 apples belonging to

the Verde Doncella cultivar was harvested. This is a commercial cultivar, highly appreciated in Spain. Apples from this batch were preclassified as ‘watercore affected’ and ‘healthy’ in the field by hydraulic sorting (Herremans et al. 2014).

After selection, the apples were transported to the LPF-TAGRALIA lab in Madrid. The next day, they were taken to the NMR facilities, and an MRI screening (20 slices per fruit) was performed on all apples to evaluate the presence or absence of watercore. Based on the screening, 12 fruits were selected (affected by different watercore levels) for 2D relaxometry experiments.

MR Equipment

All the experiments were carried out on a Biospec BMT 47/40 (Bruker, Ettlingen, Germany) operating at 4.7 T equipped with a 26-cm gradient coil and 20-cm radiofrequency probe head. It is at the Research Assistance Centre of Nuclear Magnetic Resonance, Universidad Complutense de Madrid (CEI-Moncloa) in Madrid (Spain).

Experiments

For the MRI screening, all the apples were imaged using a 2D T_2 -weighted rapid acquisition with relaxation enhancement (RARE) spin-echo sequence. Coronal images (x – z plane) were obtained from apples placed with their central axis along the y -axis of the magnet. The MRI sequence parameters were recovery time (TR) 5,000 ms and effective echo time (TE_{eff}) 62.7 ms. The field of view (FOV) was 10×10 cm², and the slices were 4 mm thick. Images were collected with 256×128 acquisition matrix size and converted to 256×256 in the reconstruction, using a zero filling. The total acquisition time was 2 min and 2 s. For each fruit, 20 slices were obtained. The voxel size was $390.6 \mu\text{m} \times 390.6 \mu\text{m} \times 4000 \mu\text{m}$.

The apples were divided into four classes according to the percentage of damaged tissue segmented in each slice of the tomography. The segmentation method chosen was the maximum entropy, which has shown favourable results in previous studies (Herremans et al. 2014). It was stated that class 0 corresponds to apples having 0 % of damaged areas; class 1 corresponds to apples slightly affected by watercore (from >0 to 10 % of affected tissue), class 2 to apples moderately affected (from >10 to 35 % of affected tissue) and class 3 to apples strongly affected by watercore (>35 % of affected tissue) (Fig. 1, Table 1). From this screening, 12 apples were selected, three belonging to each class, and then submitted to 2D relaxometry. The 2D relaxometry was based on T_1/T_2 sequence. The two types of relaxometry approaches used were global and localized.

For global relaxometry, an inversion-recovery-CPMG sequence (Hills 2010) was used. With this sequence, it is possible to obtain the probability density of different proton pools

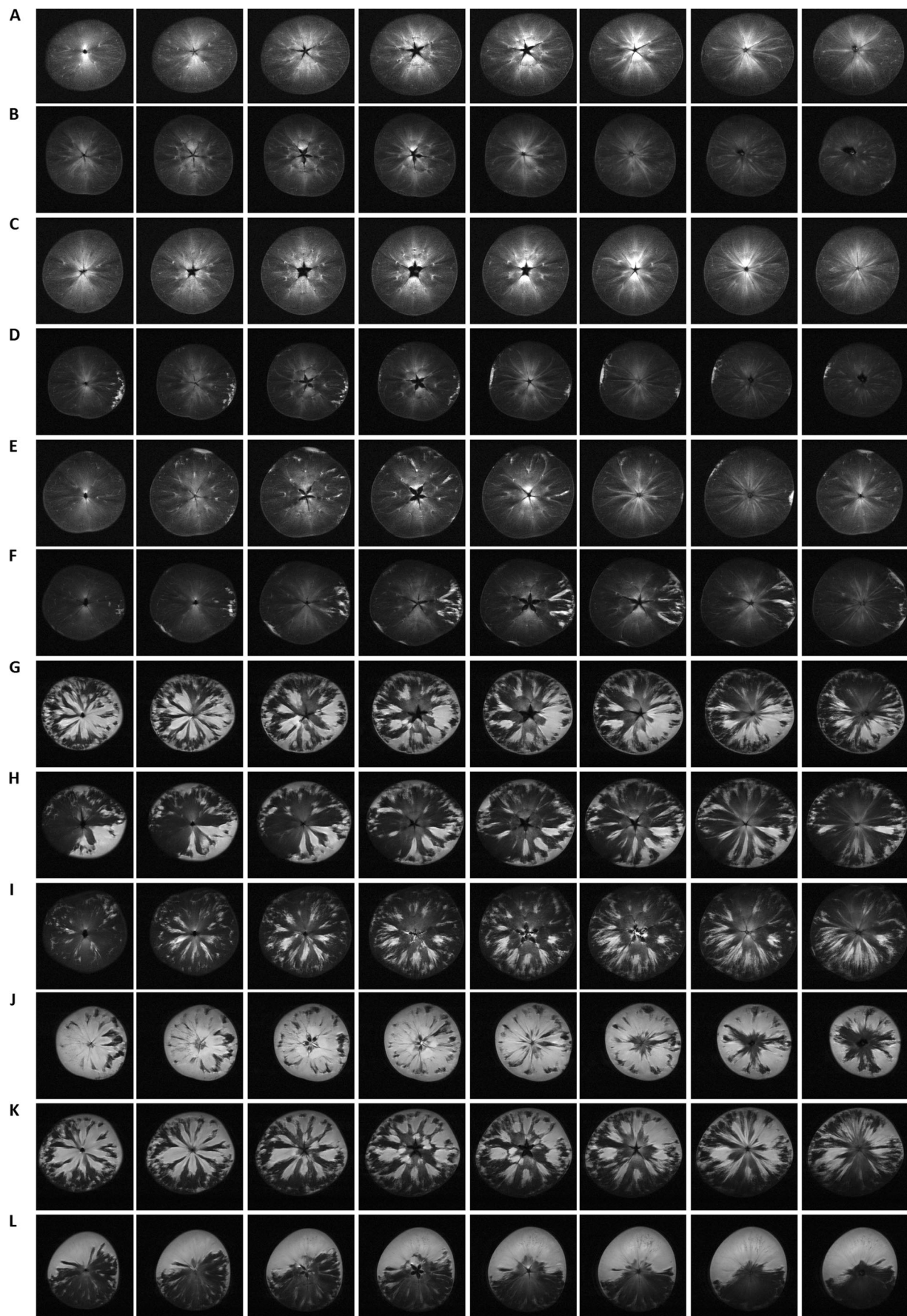


Fig. 1 MRI tomography of the 12 selected fruits. The image shows the eight central slices of the tomography of each fruit. They vary their affliction level from the top to the bottom, grouped in batches of three apples, which belong to class 0 (a–c), 1 (d–f), 2 (g–i) and 3 (j–l), respectively

distinguished based on their T_1 and T_2 values. T_1/T_2 correlation spectra were acquired with the following parameters: 64 echoes with an interpulse delay of $\tau=4$ ms. The choice of this value was based on results provided by Hernández-Sánchez et al. (2007), who found the higher differences between sound and affected tissue at those values at a high magnetic field of 300 MHz. The T_1 dimension was encoded using 64 inversion recovery steps with inversion recovery times logarithmically increased from 5 ms to 15 s; the repetition time was set to 15 s, which guarantees the total recovery of the magnetization.

For localized relaxometry, which refers to relaxometry focused in determined slices of the apples in a non-destructive and non-invasive way, an inversion-recovery-CPMG sequence with slice selection was used (Venturi and Hills 2010). The parameters for this acquisition were as follows: 64 echoes with an interpulse delay of $\tau=5$ ms, which is the lower value that we could set due to the gradients, and 64 inversion recovery steps, with inversion recovery times varied from 5 ms to 15 s, for each slice. The repetition time was 15 s, and seven different slices for each fruit were evaluated.

2D T_1 - T_2 spectra were extracted from the magnetization matrix, $M(t_1, t_2)$ using the standard 2D fast inverse Laplace transform (Callaghan et al. 2007; Song et al. 2002) on Matlab 7.0.

Table 1 Percentage of the segmentation results with respect to the total apple volume in each apple, classified by groups according to their segmentation percentage

| Apple | Mean segmentation area (%) | Class |
|-------|----------------------------|-------|
| A | 0 | 0 |
| B | 0 | 0 |
| C | 0 | 0 |
| D | 4.05 | 1 |
| E | 2.62 | 1 |
| F | 2.97 | 1 |
| G | 34.48 | 2 |
| H | 21.13 | 2 |
| I | 10.33 | 2 |
| J | 78.55 | 3 |
| K | 38.50 | 3 |
| L | 45.71 | 3 |

Class 0, 0 %; class 1, >0–10 %; class 2, >10–35 %; class 3, >35 %

Results

2D T_1/T_2 correlation spectra shown in Figs. 2, 3, 4, 5 and 6 provide quantitative information about the water compartmentation. Water in different subcellular organelles is characterized by different proton relaxation times T_1 and T_2 . Watercore disorder is due to the water movement from the intracellular (vacuole and cytoplasm) to the intercellular spaces. Thus, the highest differences between healthy apples and/or tissues (according to global or localized relaxometry) and watercore-affected apples and/or tissues were seen in water distribution, in which maximum peaks are found at around 10 and 10^{-1} -s T_2 and $10^{-0.5}$ and 10^1 T_1 values (Hills and Remigereau 1997; Hernández-Sánchez et al. 2007).

Global 2D T_1/T_2 relaxometry

Figure 2 shows the different global 2D relaxometry correlation spectra of all the apples studied from the four respective watercore affliction levels. On the healthy apples (Fig. 2a–c), water at several microscopic structures (with T_1 values between $10^{-0.5}$ and 10^1 s) can be addressed (vacuole and cytoplasm and extracellular water). A preliminary assignment can be done, according to previous studies on pear and apple tissue, respectively (Hernández-Sánchez et al. 2007; Marigheto et al. 2008), which conclude that water is compartmentalized into vacuole, cytoplasm and extracellular space. According to Marigheto et al. (2008) and Hills (2010), peak 1 is associated with vacuolar water, which has the longest T_2 times, and peak 2 and peak 3 with cytoplasm and extracellular water, with lower T_2 times. In our study, peak 3 is present in all fresh apples and all apples affected by a low watercore (class 0 and class 1, respectively) (Fig. 2a–f). Nevertheless, it only appears in one apple from class 2 (apple G) and two of class 3 (apple J and apple L).

In all the samples tested, peaks 1 and 2 appear merged (peak 1–2), and no distinction between these compartments can be done. In Fig. 2a–c, peak 3 appears split from peak 1–2. Peak 1–2 of Fig. 2a–c is associated with vacuolar and cytoplasm and extracellular water, while peak 3 in Fig. 2a–c is related to cytoplasm and extracellular water, respectively. The reduced peaks in Fig. 2b are not addressed due to a high uncertainty level. The reason why these peaks are visible only in apple B is because in the rest of the apples, the relative importance of those peaks is lower with respect to the vacuolar, cytoplasm and extracellular water.

For affected fruit (Fig. 2d–l), cytoplasm and extracellular water peak (peak 3) is present in six of the nine studied samples (apples D, E, F, G, J and L). Apples H, I and K show peak 1–2 merged with peak 3. All of those apples have a marked radial watercore pattern. This merge may be due to the varying water content of the vascular bundles that make peak 3 appear together with peak 1–2 (Fig. 2). According to Dart

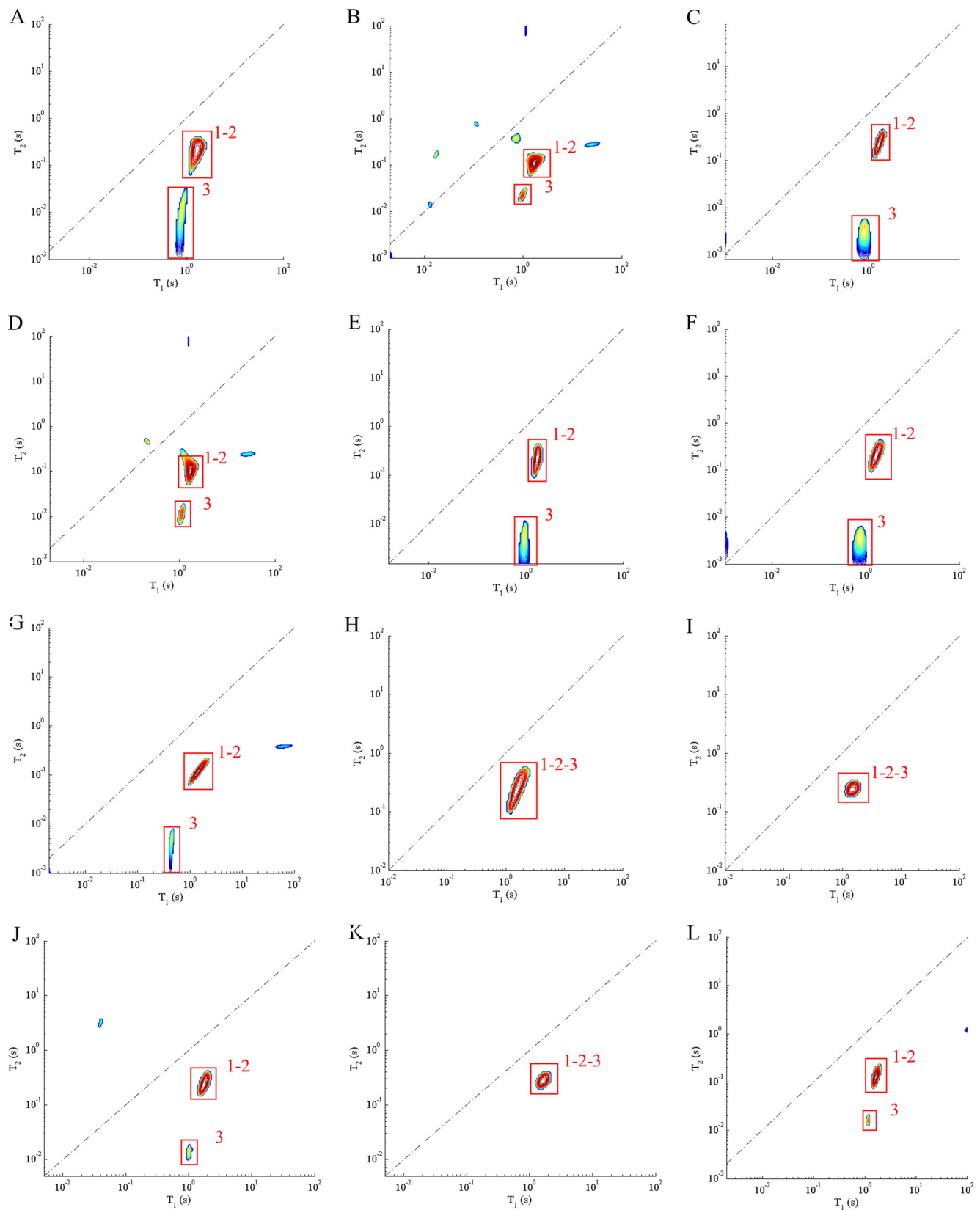


Fig. 2 Global 2D T_1/T_2 relaxometry correlation spectra, acquired at 200 MHz, of the 12 studied apples belonging to the four watercore affliction levels, with a CPMG pulse sequence. **a–c** Class 0. **d–f** Class

1. **g–i** Class 2. **j–l** Class 3. Peaks are assigned as 1 for vacuole and 2 and 3 for cytoplasm and extracellular water

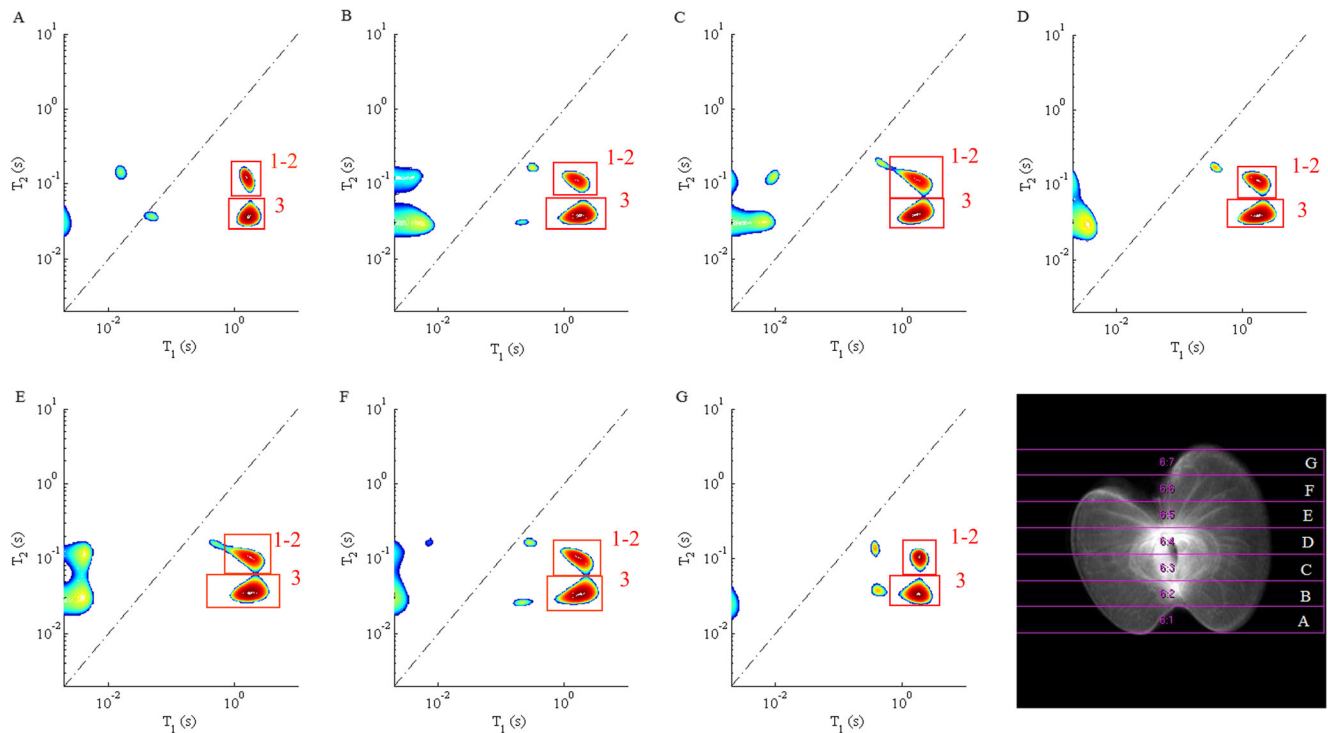


Fig. 3 Localized 2D T_1/T_2 relaxometry in seven slices (a–g) in a healthy apple. Peaks are assigned as 1 for vacuole and 2 and 3 for cytoplasm and extracellular water

and Newman (2005), radial watercore is typical of ripe fruit. It can be compared with the results obtained by Marigheto et al. (2008), who analyzed a piece of vascular tissue extracted from a very ripe apple. In their T_1/T_2 cross-correlation spectra, they

did not find peak 3 due to the varying water content of the vascular bundles.

As shown in Table 2, the average value of the maximum T_1 is shorter in apples affected by watercore (class 2 and class 3).

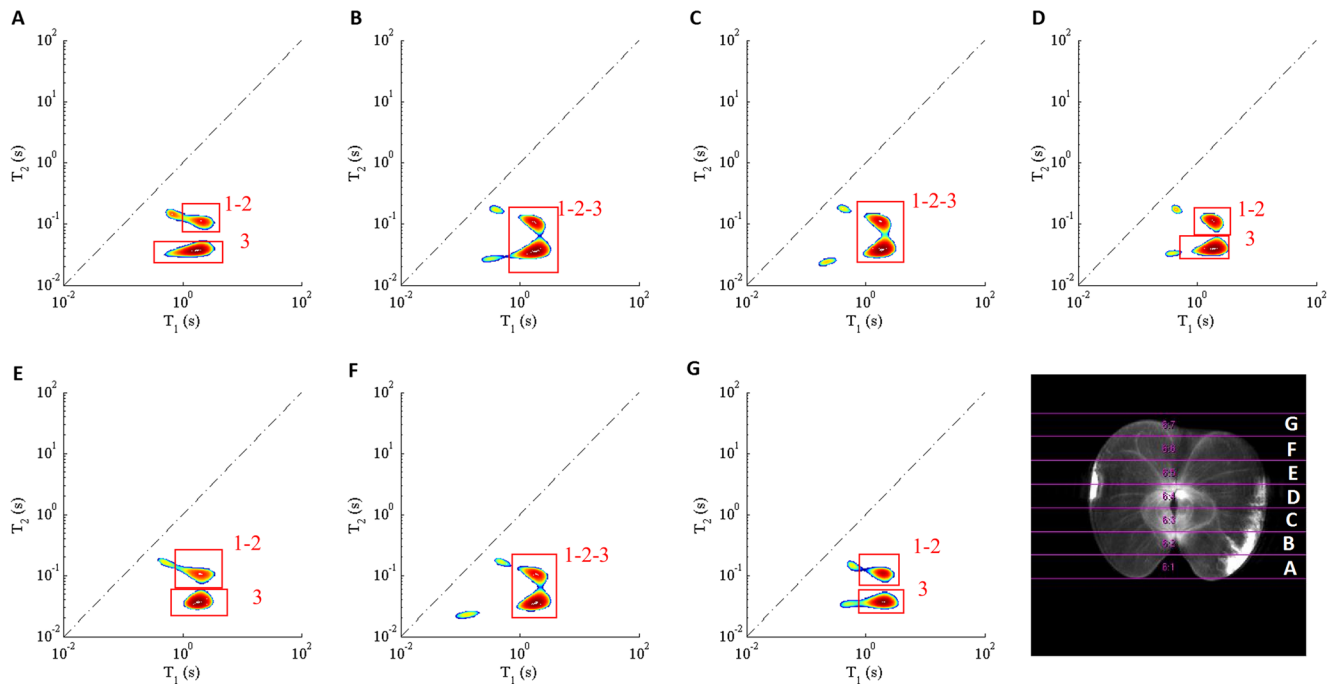


Fig. 4 Localized 2D T_1/T_2 relaxometry in seven slices (a–g) in an apple belonging to class 2 (0–10 % of affected tissue). Peaks are assigned as 1 for vacuole and 2 and 3 for cytoplasm and extracellular water

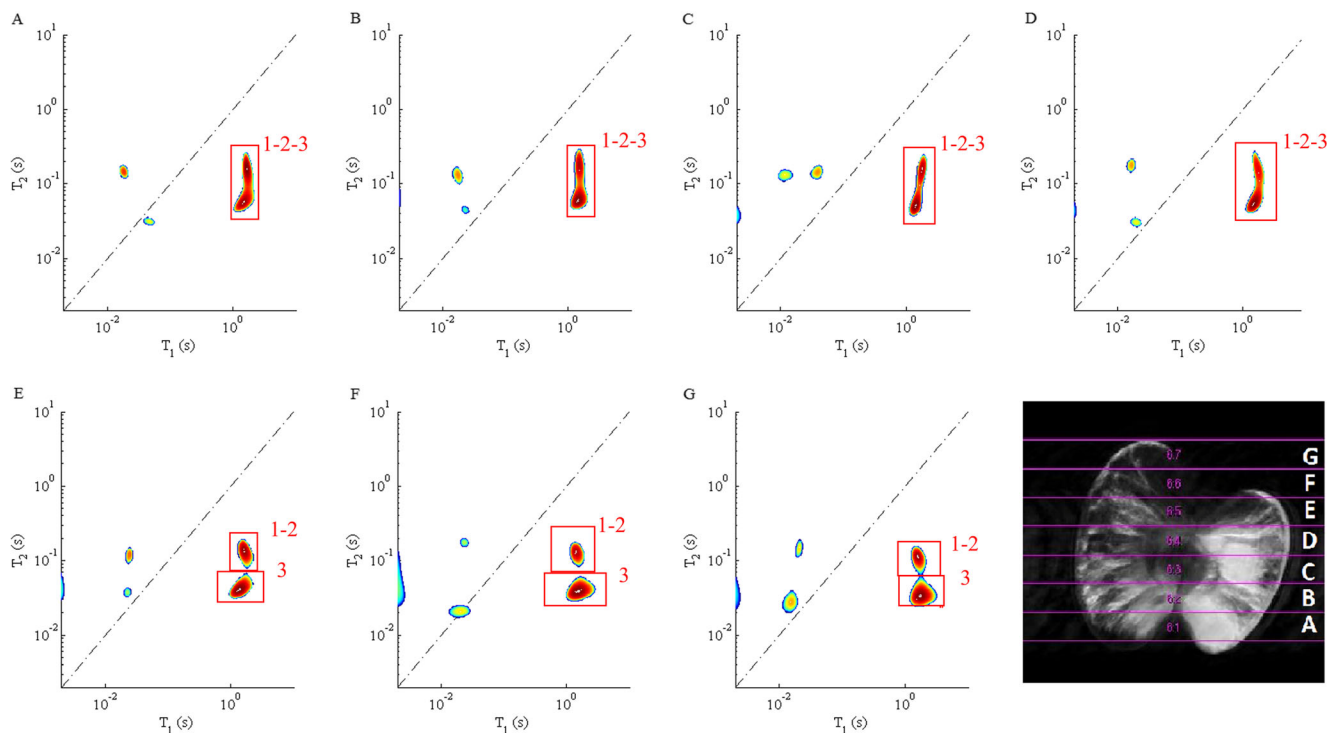


Fig. 5 Localized 2D T_1/T_2 relaxometry in seven slices (a–g) in an apple belonging to class 3 (10–35 % of affected tissue). Peaks are assigned as 1 for vacuole 2 and 3 for cytoplasm and extracellular water

However, the distribution is broader in very affected apples (class 3) as reflected in the standard deviations shown in Table 1. For the average values of T_2 , the behaviour is different. It shows longer values for class 3 at peak 1–2, with a broader distribution. Nevertheless, for peak 3 cytoplasmatic and extracellular water, the behaviour looks different: Shorter T_2 is found for class 3. These results contrast with measurements at lower magnetic fields in which the value of the main T_2 water peak increases with watercore (Cho et al. 2008). This may be a result of susceptibility effects, as exposed by Marigheto et al. (2008).

Localized 2D T_1/T_2 relaxometry

Results on localized 2D T_1/T_2 relaxometry show that there are intraapple differences depending on the slice of study. Figure 3 shows a healthy apple, with seven slices of study. In general, four proton peak populations can be distinguished, although only two are addressed. Peak 1–2 corresponds to vacuole and cytoplasmatic-extracellular water, respectively; peak 3, again, is assigned to the cytoplasmatic-extracellular water (Marigheto et al. 2008). It is important to state that in the regions belonging to the core of the apple (regions C, D and E of Fig. 3), a particular behaviour can be found. Here, peak 3 (assigned to the intercellular water) experiences an approach to Peak 1–2 due to a variation in tissue composition compared to other regions. This is due to the heterogeneity found within this region, having lower porosity, higher moisture content

and larger pore size and thus higher water mobility. The equatorial region of the apple is characterized by the presence of more abundant vascular tissue (Defraeye et al. 2014; Verboven et al. 2008; Mendoza et al. 2010), and it will result in variable water content in the vascular bundles that will be reflected in the proton peak population.

Figure 4 shows a fruit from class 1 (>0 to 10 % of affected tissue). In this case, peak 1–2 and peak 3 appear merged in slices B, C and F, which correspond to those slices that have watercore damage. Figure 5 shows a fruit belonging to class 3 (from >10 to 35 %). On slices A to D, peak 1–2 and peak 3 appear merged. This is due to the water mobility that occurs in watercore disorder from the inner of the cells to the intercellular spaces. In regions E–G, where the damage is lower, Peaks 1–2 and 3 appear separated. Again, in the core tissue (regions B, C and D), the behaviour of some unassigned peaks seems to be of particular nature, which may be due to changes in tissue composition and to the presence of seeds. In regions E, F and G, where the damage is shallow, peaks 1 and 2 appear separated.

Figure 6 shows three strongly watercore-affected apples (more than 35 % of damaged tissue), with different watercore patterns. The three show that vacuolar and cytoplasmatic and intercellular water (Peak 1–2) appears merged with cytoplasmatic and extracellular water (peak 3) in regions of watercore disorder. Individual peaks cannot be assigned specifically to one compartment, so this may be the result of a diffusive exchange regime, from the inner part of the cells and

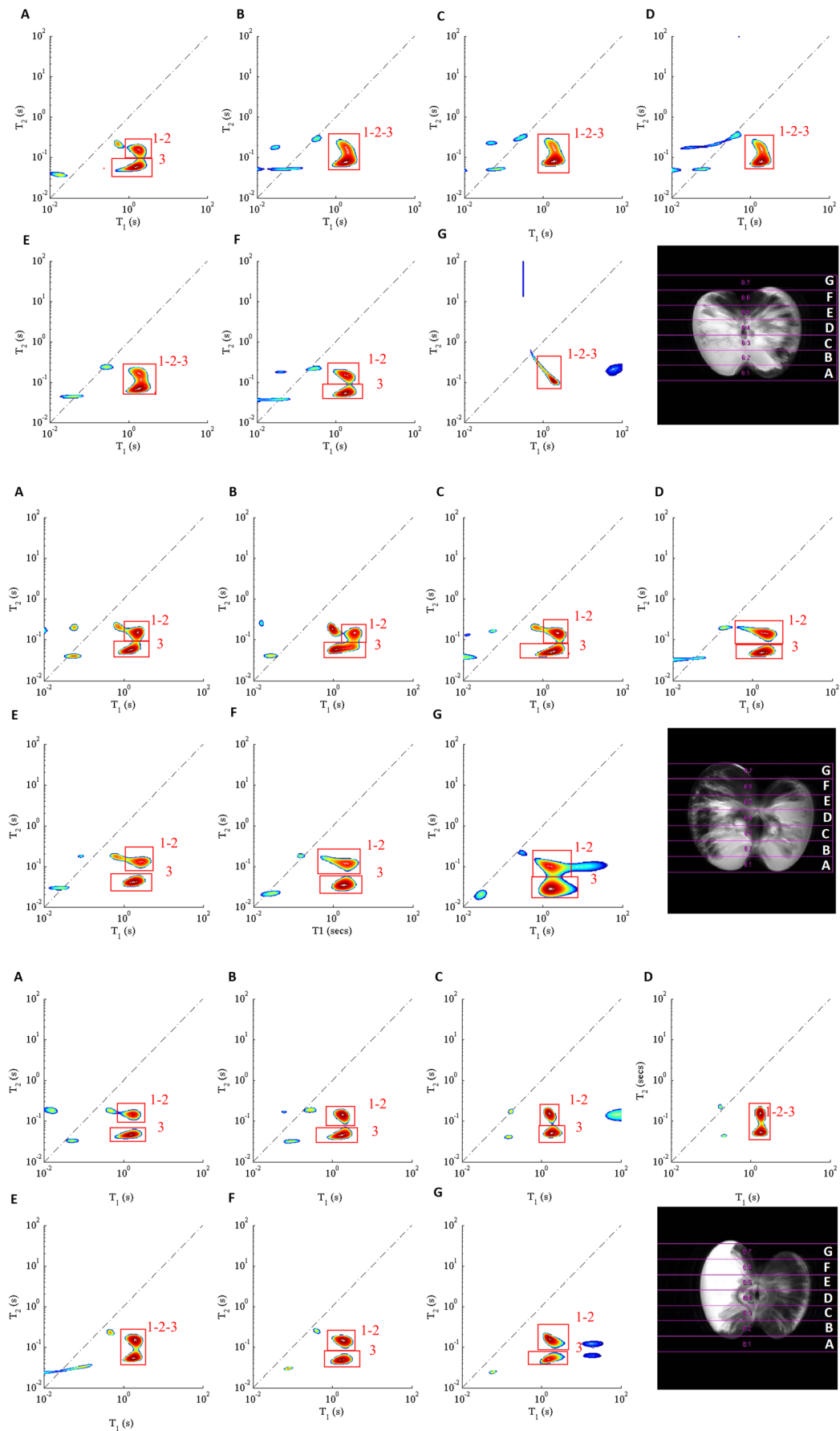


Fig. 6 Localized 2D T_1/T_2 relaxometry in seven slices (a–g) in three strongly watercore-affected apples (>35 % of affected tissue), with different watercore patterns. The *apple at the top* has 78.55 % of affected tissue. The *apple at the centre* has 38.50 % of affected tissue and a marked radial watercore pattern. The *apple at the bottom* has 45.71 % of affected tissue and a much localized damage region. Peaks are assigned as 1 for vacuole 2 and 3 for cytoplasm and extracellular water

its different compartments to the intercellular spaces. Slices A and G have not only tissue but also air. This may affect the results in these slices. The fruit on the top of the figure, which has 78.55 % of affected tissue, has all the slices of study affected by strong watercore, and thus, no individual assignment can be made to each individual compartment. The apple at the centre of the figure, with 38.50 % of affected tissue, presents a marked radial watercore pattern. As before, in slices where watercore affliction is higher, vacuolar, cytoplasm and extracellular water appears merged, while in slices where the affliction is lower, these peaks appear split. For the fruit at the bottom of the figure, which has 45.71 % of affected tissue and that presents a different watercore pattern, the relaxometry behaviour is slightly different. We can see that the damaged region is located in a very specific area. Slices from B to E show the mixture of water from the intracellular to the extracellular compartment. Nevertheless, slices A and G have not only tissue but also air, so the results in the relaxometry can be uncertain.

Discussion

Global relaxometry did not show a huge difference on the peak distribution between sound and watercored apples. This is due to the fact that the fruit has both microscopic and macroscopic heterogeneity, which suggests that global

Table 2 Average maximum values with standard deviation of the water proton relaxation times of different peaks from T_1 and T_2 correlation spectra of the four classes of ‘Verde Doncella’ entire apple at 200 MHz with an inversion-recovery-CPMG sequence

| | T_1 (ms) | T_2 (ms) |
|---|------------------|--------------|
| Vacuole+cytoplasm and extracellular water: peak 1–2 | | |
| Class 0 | 1,693.3 (75.0) | 273.3 (5.7) |
| Class 1 | 1,780.0 (22.9) | 265.6 (12.5) |
| Class 2 | 1,525.6 (2.3) | 271.5 (7.6) |
| Class 3 | 1,073.3 (110.15) | 297.5 (14.9) |
| Cytoplasm and extracellular water: peak 3 | | |
| Class 0 | 863.3 (40.41) | 20.1 (18.1) |
| Class 1 | 966.1 (173.3) | 12.9 (11.89) |
| Class 2 | 128.6 (222.8) | 3.6 (6.3) |
| Class 3 | 718.6 (624.1) | 16.0 (16.5) |

relaxometry will provide an average relaxation spectrum of the whole fruit (Venturi and Hills 2010). Nevertheless, some qualitative and quantitative information can be achieved from the peak distribution and T_1 and T_2 values.

Apples having a very marked radial watercore pattern do not have a separated peak 3, belonging to cytoplasm and extracellular water. This may be due to the merging of peak 1–2 and peak 3 due to the high variation of water content of the vascular bundles (Marigheto et al. 2008).

In other pathologies, such as mealiness and internal browning, T_2 values, observed in the high magnetic field, show a decrease compared to sound tissues (Barreiro et al. 2002; Hernández-Sánchez et al. 2007). This is due to the microscopic diffusion produced by the magnetic susceptibility changes when the integrity of the cellular membranes is affected. In the case of watercore, a light increase in the T_2 values is observed in vacuolar and cytoplasmatic water, which suggests that for watercore disorder, the cellular membrane integrity is not affected, as what happens in mealiness and internal browning. Furthermore, there is a huge liquid amount, which means a higher proton concentration that means a higher T_2 value. In strongly affected fruit, a slight decrease in T_2 values from the cytoplasm and extracellular water is observed. This agrees with the observations made by Cho et al. (2008), who found a slight decrease in T_2 values from extracellular and cytoplasmatic water in moderate and severe watercore-affected fruit. This can be caused by a potential internal breakdown or internal browning developing in very severe watercore cases (Bennedson and Peterson 2005). Furthermore, previous studies in mealy apples showed longer T_1 values with respect to sound apples (Marigheto et al. 2008). In mealy apples, the mechanism is almost opposite from that which occurs in watercore: As mealiness develops, the cells become more separated, and the diffusion of water between compartments becomes slower than in the fresh fruit (Marigheto et al. 2008). Nevertheless, in watercore apples, the diffusion of the water between compartments increases as watercore develops. Thus, a decrease of T_1 values is observed on vacuolar, cytoplasmatic and extracellular water, contrary to that observed by Marigheto et al. (2008) in mealy apples.

Localized relaxometry allows the study of specific slices in a non-destructive way. It is interesting especially for samples having micro- and macroscopic heterogeneity, which is the case for apple fruit. In the particular case of watercore, which may develop different patterns (block and radial) (Clark et al. 1998; Harker and Watkins 1999; Melado-Herreros et al. 2013a, b), it is especially useful. For localized relaxometry, a clear difference was found as sound apples showed neat peaks for water at different subcellular compartments, such as the vacuole and cytoplasm and extracellular compartment, while in watercore-affected tissues, such compartments appear merged, which implies the mixture of vacuolar and cytoplasmatic and extracellular water. This means that there

exists a diffusive exchange regime between the inner cell compartments and between the inner part of the cells and the intercellular spaces. This agrees with studies performed by Yamada et al. (2006, 2012), who found that in watercored apples, the permeability of the tonoplast increases especially to sorbitol, and they also found a higher permeability of the plasma to other sugars, such as fructose, glucose and sorbitol. This means that if there is an increase of the permeability of the membrane, diffusion between different compartments will be easier.

Furthermore, the mixture of intracellular water with intercellular water suggests that intercellular spaces are filled with fluid, as what is typical from watercore disorder (Suzuki et al. 2002). This agrees with the study performed by Herremans et al. (2014) in which they observed that the pores between cells in watercore-affected tissues are almost absent, thus likely filled with water, though the mechanism of the redistribution of the water in watercore-affected fruit is still unclear (Yamada et al. 2012). Also, Cho et al. (2008) found an increase of water in the extracellular spaces and the cytoplasm of watercore-affected apples and a decrease of the water from the vacuole, which is consistent with the observation that some of the intercellular air spaces are filled with fluid (Marlow and Loeschner 1984; Herremans et al. 2014).

According to Marigheto et al. (2008) and Hills (2010), peak 3 is associated with the cytoplasm and extracellular compartment. It means that peak 3 is formed by cytoplasm and extracellular water, though it is impossible to know which is the proportion of each one. Marigheto et al. (2008) and Hills (2010) also found the presence of this peak in healthy apple, which means that on healthy apples, there exists a small proportion of extracellular water. In the work carried out by Herremans et al. (2014), they observed a reduction in the porosity in the affected cases, from 30 % (in healthy tissues) to the 7 % (in watercore-affected tissues). However, using X-ray CT, it was not possible to detect extracellular water, which suggests that this type of water is present in a very small amount in the extracellular space of healthy tissues.

Conclusions

In this study, 2D T_1/T_2 relaxometry is a feasible non-destructive technique to assess microstructure (<100 μm) inside a whole fruit in a non-destructive way. Nevertheless, for samples having micro- and macroscopic heterogeneity, such as actual food, localized relaxometry allows addressing predefined slices of tissue to understand specifically the microstructure state of a particular region of the fruit. In the case of watercore disorder, this is very useful for determining the level of affliction of a specific region of a fruit, to perform a study of different watercore patterns (block and radial) and to perform a detailed microstructure study of specific fruit

regions without destroying the sample. Localized relaxometry provides more exhaustive information, which cannot be derived from global 2D T_1/T_2 relaxometry, since it allows the study of a specific region. Therefore, the combination of localized 2D T_1/T_2 relaxometry with MRI, it is feasible to perform a non-destructive multi-scale study of watercore development. This technique is applied to intact fruit for the first time in the literature.

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